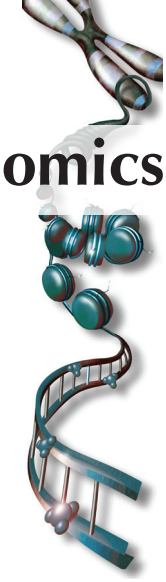
Special Report

For reprint orders, please contact: reprints@futuremedicine.com

Epigenomics



Transgenerational latent early-life associated regulation unites environment and genetics across generations

The origin of idiopathic diseases is still poorly understood. The latent early-life associated regulation (LEARn) model unites environmental exposures and gene expression while providing a mechanistic underpinning for later-occurring disorders. We propose that this process can occur across generations via transgenerational LEARn (tLEARn). In tLEARn, each person is a 'unit' accumulating preclinical or subclinical 'hits' as in the original LEARn model. These changes can then be epigenomically passed along to offspring. Transgenerational accumulation of 'hits' determines a sporadic disease state. Few significant transgenerational hits would accompany conception or gestation of most people, but these may suffice to 'prime' someone to respond to later-life hits. Hits need not produce symptoms or microphenotypes to have a transgenerational effect. Testing tLEARn requires longitudinal approaches. A recently proposed longitudinal epigenome/envirome-wide association study would unite genetic sequence, epigenomic markers, environmental exposures, patient personal history taken at multiple time points and family history.

First draft submitted: 6 October 2015; Accepted for publication: 11 December 2015; Published online: 7 March 2016

Keywords: aging • childhood • development • epigenetics • experiences • insult • intergenerational • late life • neurodegenerative • nutrition • post traumatic

Debomoy K Lahiri*[,1,2], Bryan Maloney[1], Baindu L Bayon[2], Nipun Chopra[1], Fletcher A White[3], Nigel H Greig[4] & John I Nurnberger[1,2]

[1]Department of Psychiatry, Stark Neurosciences Research Institute, Indiana University School of Medicine, 320 West 15th Street, Indianapolis, IN 46202, USA

[2]Department of Medical & Molecular Genetics, Indiana University School of Medicine, 320 West 15th Street, Indianapolis, IN 46202, USA

[3]Department of Anesthesia, Stark Neurosciences Research Institute, Indiana University School of Medicine, 320 West 15th Street, Indianapolis, IN 46202, USA

[4]Translational Gerontology Branch, National Institute on Aging, NIH, Baltimore, MD 21224, USA

*Author for correspondence:
Tel.: +1 317 274 2706
Fax: +1 317 231 0200
dlahiri@iupui.edu

Background

Recent human genome research, accompanied by ever-increasing technological developments, continues to significantly enhance our understanding of human diseases in terms of etiology, diagnosis, drug response and novel treatment avenues. Diseases are often heterogeneous (several diseases overlapping as one) and multigenic, meaning disease phenotype (symptoms) can be caused by multiple genes/mutations, although each individual might or might not have one main independently causal rare variant. Exclusive use of homogeneous populations (e.g., Canadian founder, Icelandic and Utah cohorts) in studies will not yield clinically relevant biomarkers applicable to the population at large.

In the present Special Report, we will briefly mention the advances and limits of current genetic research and technology and then focus onto gene–environment interaction that would support and expand various genetic models to explain human diseases. Our premise is that sporadic cases of Alzheimer's disease (AD), Parkinson's disease (PD), suicide and other disorders display well-established environmental associations. The latent early-life associated regulation (LEARn) model integrates early-life events, environmental exposures and gene expression while providing a mechanism for disorders occurring later in the life of an organism. Here, we propose that this process can also occur across generations via transgenerational LEARn (tLEARn). We provide evidence from different research studies in favor of the proposed concept, tLEARn, which uniquely interconnects environmental

Future Medicine part of fsg

influence upon genes via epigenetic modification due to early-life 'hits', with the epigenomic record of such 'hits' being passed along to further generations.

Genomic variations: small variants, copy number variants & rare variants do not add up to explain the cases of sporadic diseases

Human genomes are usually considered a spectrum of 'normal' variation within an ethnically diverse population. A small fraction of variants can be identified, which may be unique to a disease or drug profile. Personalized medicine would be based on defining genetic subgroups by rare genetic variants. According to the common variant hypothesis, which postulates that gene variation within the human founding population underlies the predisposition to heritable diseases, most SNPs have little clinical value.

At best, most SNPs discovered in genome-wide association studies (GWAS) associate with slight increases in disease incidence and are often described as 'predisposition', 'risk' or 'susceptibility' factors. The research value of these changes points toward biochemical pathways of interest, particularly to discover intervention targets that are perturbed by environmental factors under the reasonable presumption that a genetic risk mimics the effect of environmental damage upon that gene's normal expression. This has been the case in the use of statins in response to genetic discoveries regarding HMG reductase to reduce low-density lipoprotein cholesterol [1]. Furthermore, cumulative effects of small genetic changes may produce great overall variability in psychiatric conditions like bipolar disorder [2].

Genome-wide biomarker discovery platforms systematically reveal rare genetic variants that could be used in combination to differentiate responders from nonresponders and to predict serious adverse events in drug development trials by shared biochemical complexes in which these genes participate. Furthermore, while copy number variants (CNVs) and other structural variants make a major contribution to genetic variation, they are frequently found in both healthy people and those with sporadic diseases, such as autism spectrum disorder (ASD), schizophrenia and PD. Recently, the importance of *de novo* (occurring for the first time in an individual) mutation in disease etiology has been recognized [3,4]. Rare genetic variants have recently been found to play a larger role in diseases than previously known – up to 7% of cases of bipolar disorder, schizophrenia or ASD [5]. This could potentially be of aid to millions of people, depending on overall disease prevalence. On the other hand, a model that explains up to 7% of cases still does not explain over 90% of cases, and in some situations, such as ASD with IQ ≥100, *de novo* mutation has no correlation [3].

Genetic models can be greatly supported and expanded by explicit mechanistic explanations of gene–environment interaction. For example, well-established environmental associations have been found for PD [6], AD [7,8], suicide [9] and other disorders [10,11]. A model is needed that incorporate the role of environment, including both early and later-life exposures, the resultant 'somatic epitypes', their effects on adult-life disorders and potentially beyond a single lifespan. Such a model would provide a unique concept, tLEARn – the major focus of the present Special Report. This concept will interconnect environmental influence upon genes via epigenetic modification due to early-life hits with the epigenomic record of such hits being passed along to further generations.

Genetic variation on its own does not explain all pathogenesis of 'sporadic' disorders

The origin, pathogenesis and trajectory of idiopathic or sporadic diseases, including some forms of neurodegenerative disorders, that do not follow the Mendelian law of inheritance have attracted increasing attention in recent years, particularly in developing models of polygenic inheritance, endophenotypes, *de novo* mutation and others. Large-scale genomic association studies have pointed toward pathway disruptions that could occur at any point, not just single gene [12]. Nevertheless, in some disorders with known genetic components, such as AD, cases that can be explained solely by genetic mutation are a minor fraction. Those forms of AD that are autosomally inherited and traceable to specific DNA variations account for no more than 10% of cases [13]. Primary DNA sequence variation certainly accounts for substantial proportions of variance for some diseases, but environment and gene × environment interaction can account for a greater proportion in others. Epigenetics would be no panacea. The epigenetic approach would be an additional tool to be used when appropriate for a specific disorder.

There is an important role played by epigenomic factors in conditions as diverse as AD [14–16], PD [16–18], ASD, schizophrenia and even suicide [19–21]. The field has determined epigenetic changes for specific genes, such as *SHANK3* [22], brain region-specific epigenetic alterations [23] and several differences in the epigenome between monozygotic (MZ) twins discordant for ASD [24]. This epigenomic discordance is of particular interest because there is not a simple epigenome-wide difference between ASD and non-ASD MZ twins. Instead, differential methylation was clustered around specific loci, including *NLGN2, SNRPN* and ATP-sensitive inward rectifier potassium channel 10 (*KCNJ10*), among others. In addition, further site-specific methy-

lome differences were found to depend upon whether ASD was familial or sporadic [25–27]. In AD, the epigenomic evidence is as direct as brain cortical neuron-specific differences in gene promotor methylation in twins discordant for AD [28] and overall hypomethylation and hypohydroxymethylation of DNA from hippocampus of AD patients versus non-AD subjects [29]. However, epigenetic effects on neuropsychiatric issues are not simply a matter of insufficient DNA methylation. In fact, brains from victims of suicides show increased methylation [19,21]. Bipolar disorder is also associated with increased DNA methylation and gene repression, including *KCNQ3* [30–32]. *BDNF* was subject to hypermethylation and downregulation in major depressive disorder [33,34].

However, DNA modification is not the sole form of epigenomic marker. The other major epigenomic marker is the modification of histones, including acetylation, phosphorylation and ubiquitination, among others [35]. Aberrant histone acetylation is associated with AD [14] and post-traumatic stress disorder (PTSD) [7]. Exposure to the pesticides paraquat and dieldrin are associated with PD [36]. Dieldrin and other pesticides also induce hyperacetylation of histones H3 and/or H4 [37,38], and such induced hyperacetylation is strongly associated with dopaminergic neurotoxicity [6,39]. Of particular interest, psychiatric (antidepressant) effects have been noted for epigenetic drugs, particularly the histone deacetylase inhibitor MS-275 [40]. In addition, nicotine, heroin, methamphetamine, cocaine and ethanol each induce chromatin-altering modifications of histones when administered through methods typical of their abuse. These alterations are followed by changes in multiple pathways in brain reward circuitry [41].

Epigenomic contributions to neurobiological disorders may actually reach across generations. For example, an association has been found between paternal age at conception and risk for ASD and schizophrenia in offspring [42–44]. While one interpretation has been an accumulation of germ-line DNA mutations, an alternate mechanism would be an accumulation of environmentally induced epigenomic changes. The connection between environmental stress and epigenomic changes is well known [45–47]. The idea that prenatal and perinatal conditions influence health in childhood and later life has been long established. That the life of the father may also exert an influence adds another level of complexity.

A molecule that has been getting a good deal of attention in the literature is miRNA, which is short, noncoding RNA capable of modulating protein expression by acting as a set of specific recognition site 'sockets' in the RISC translation regulation complex, primarily targeting the 3'UTR of associated mRNA [48]. Modulation of various epigenetic markers requires miRNA activity in *Arabidopsis* [49] and has been shown to exist in mouse models [50] and clinical samples [51]. miRNA-mediated methylation [49,52–54], acetylation [50] and ribosylation [55] have been reported in the literature – primarily related to tumorigenesis. Thus, while miRNA plays 'a role' in epigenetics, such a role would be, therefore, analogous to *DNMT* or *HDAC* or proteins that regulate *DNMT* or *HDAC* and does not grant miRNA a special status as an epigenetic agent. Environmental factors such as cigarette smoke [56], physical activity [57], metal exposure [58] and infection [59] can regulate miRNA levels. The miRNA genes can be under the influence of epigenetic modification [60–62]. We do not claim that miRNA is specifically inherited as an epigenetic marker or any sort of particularly epigenetic factor, any more than any other DNA transcription product.

LEARn unites environmental exposures & gene expression while providing a mechanistic underpinning for later-occurring disorders

The LEARn model encompasses environment–genome interaction in the etiology of sporadic neuropsychiatric disorders. Such disorders include, but are not limited to ASD, affective disorders, neurodegenerative disorders such as AD and PD, and others. The model connects environmental influence to biological outcomes through the mechanism of altering biochemical (such as epigenetic) markers. In short, LEARn posits that, for many sporadic disorders, the body accumulates 'hits' (that may include some genetic predispositions), but no single hit is sufficient to cause disease, instead, each is individually dormant. Many hits are of environmental origin and are 'recorded' and maintained in the organism through epigenomic markers (Figure 1). If a sufficient number of 'correct' hits occur before a critical developmental/aging threshold, the organism will develop the corresponding disorder [63,64]. The critical elements of the LEARn model are latency and that the latent hits can affect the organism even if the specific (causative) stimulus has long since been cleared, since the immediate biochemical effect is a persistent epigenomic alteration. LEARn-mediated disorders are not immediate acute products of a malign stimulus ASD, AD, PD or others would not appear until a critical threshold of hits is reached, and this critical threshold may include normal biological stages of development, maturation and aging. This is similar to the 'n-hit' model of oncology [63,65].

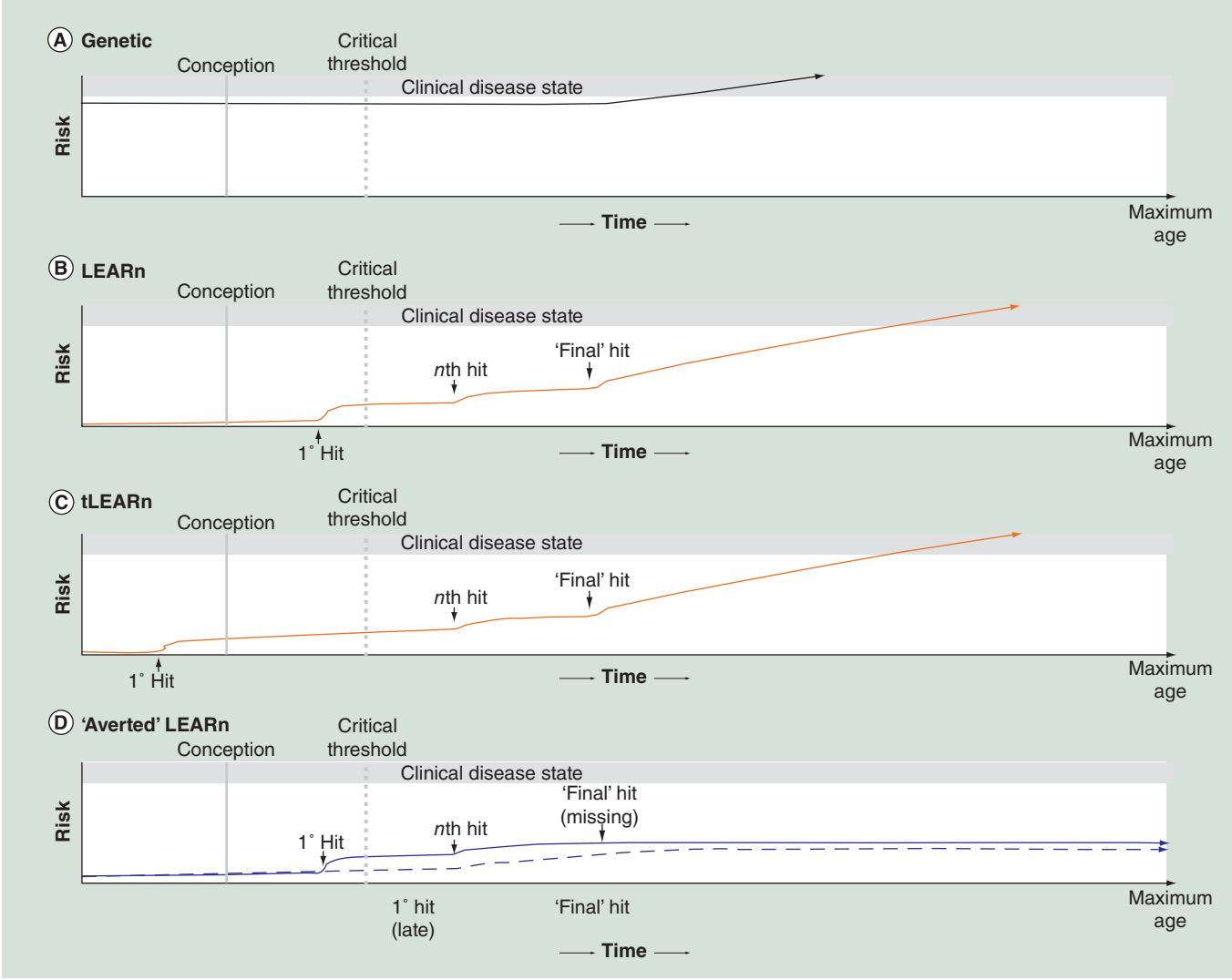


Figure 1. The latent early-life associated regulation model. LEARn explains idiopathic disorders on the basis of accumulation of 'hits' through an organism's lifespan. Hits can be environmental, genetic or epigenetic. **(A)** Genetic pathway: a 'purely' genetic disorder, such as Alzheimer's disease (AD) due to APP KM670/671NL (Swedish) mutation. AD-associated genes' DNA sequence variant determines disease state in an autosomal dominant fashion ('familial' AD/FAD). **(B)** Development of diseasevia LEARn pathway. A primary 'hit' between conception and a critical developmental threshold instills a latent epigenetic change. If this is followed by subsequent 'hits' later in life, accumulated risk factor effects reach clinical disease state as in the sporadic AD. **(C)** LEARn pathway across generations: t-LEARn. Similar to LEARn progression except that the original 'hit' has occurred in an earlier generation and been transmitted asymptomatically by epigenetic inheritance. **(D)** Remediation pathway or 'Averted' LEARn. Given that many epigenetic marker states can be altered by environmental factors, including nutrition and drugs, the possibility exists that one or more of the effects of a given 'hit' may be reversed by these means. Should this occur,accumulated effects will not reach clinical disease.
LEARn: Latent early-life associated regulation; tLEARn: Transgenerational latent early-life associated regulation.

A model uniting environment, organism & generations: tLEARn

The original LEARn model explicitly unites 'environment', described in whole or part as 'envirome' [66,67], 'exposome' [67,68], and so on, and the organism 'as an information network' [63]. The envirome is essentially 'anything outside the organism that can influence its function through epigenetic changes'. It includes sociocultural effects (poverty, education and status) and historical events (warfare and economic cycles), as well as nutrition and exposure to toxins, radiation or pathogens (sometimes called the exposome), and even elements of development and aging, which are not entirely governed by internal programs (Figure 2). From therapeutic and preventative standpoints, many aspects of the envirome are highly influential but of secondary usefulness, usually because of presumed difficulty inherent in controlling those particular elements. Two parts of the envirome that receive a great deal of medical attention are exposures to 'toxic' mate-

rials (including radiation) and nutrition. In particular, nutrition holds out potentially powerful 'handles' because it is one factor that could hypothetically be tailored and managed at the individual level.

An organism in interaction with the envirome (Figure 3) can be seen to exist on two levels. Physically, it is the information coding and transmitting molecules within an organism. These include nucleic acids (DNA and RNA) and the chemical modifications made to them (e.g., methylation and oxidation); the histones and their modifications (e.g., acetylation and phosphorylation); the proteome, which has its own primary amino acid sequence chemical modifications; and spatial (re)organization of any or all of these biochemical elements. An organism can also be modeled as an information network that 'resides' in the 'media' of the various molecules (and their 3D structures), and this changes over time, a highly dynamic system that includes both relatively stable (chromosomal DNA) and ephemeral (RNA, proteins and epigenetic markers) physical elements, together with factors that are not physical objects, but are how the objects are arranged at a given moment. It is constantly under modification in response to environmental influences. An organism can also be seen as the substrate of the envirome [66,67]. In addition, each individual organism (person) can exert influence to a greater or lesser degree upon its individual envirome and larger shared enviromes, thus influencing other organisms (people). Within LEARn, it is an environmental activity upon the organism throughout the lifespan that gives rise to idiopathic disorders. Transgenerational (tLEARn) extends this concept across generations.

Nongenetic 'intergenerational transmission' of traits such as 'harsh parenting' [69] and domestic violence [70] is documented through purely behavioral explanations, as have behavioral prevention [71] or mitigation [72,73] of such intergenerational social etiology. Nevertheless, excesses of behaviorist presumptions and haste to draw simple environmental connections have, in the past, given rise to now thoroughly discredited claims, such as the 'refrigerator mother' etiology of ASD [74], the 'aluminum hypothesis' of AD [75] or the 'schizophrenogenic mother' of schizophrenia [76], among many other discarded overly enthusiastic environmental hypotheses. The failure of such discarded hypotheses has helped give rise to a greater caution with regard to any type of 'intergenerational transmission' of specific disorders (as opposed to specific behaviors) that does not rely entirely or primarily upon strict genetic inheritance.

The heritability of genetic conditions and risks is not currently questioned. It is not asked if it is possible for traits or conditions to be inherited, only to what degree a trait is heritable. Under the classic 'central dogma', that environmental influences could be inherited beyond those that manage to change germline DNA was unthinkable. Recent work has since introduced more flexibility in so-called purely biological traits. For example, glucose intolerance can be induced by neonatal overfeeding. This intolerance does not alter

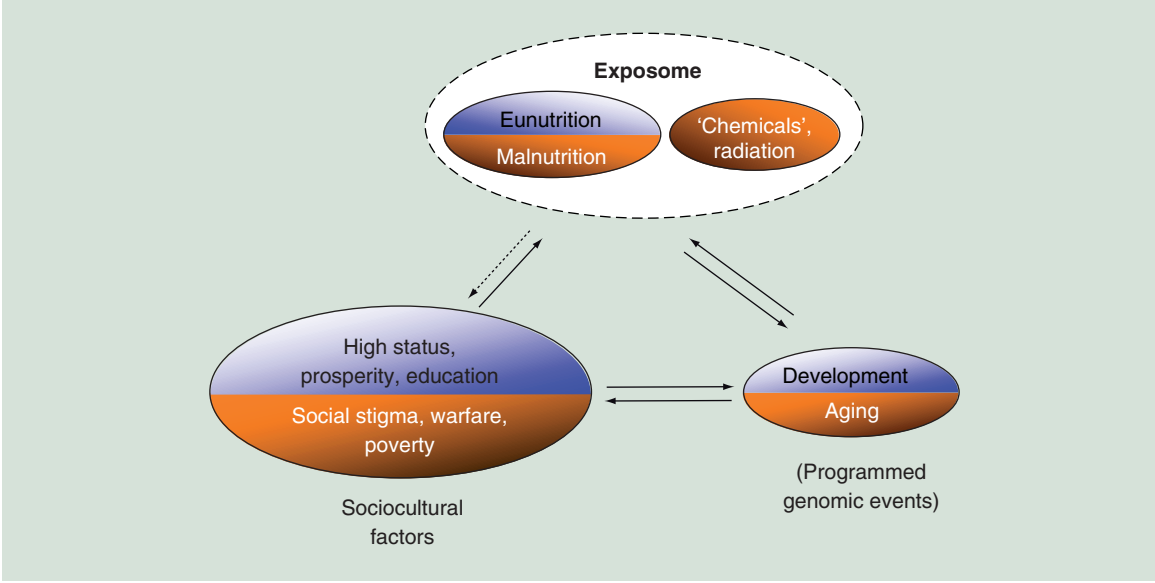


Figure 2. The envirome. The envirome is essentially everything external to an organism, including, but not limited to nutritional factors (malnutrition vs nutrition); sociocultural and historical factors, such as economic and social status, famine and warfare; exposure to 'chemicals' and radiation; and the effects of existing through time (development and aging). Nutrition and exposure to 'chemicals' and radiation can be seen as a general 'exposome'. Each of these can influence the others to some degree. Sociocultural factors exert very strong effects on the exposome, particularly in nutrition, but also in providing protection (or lack) against hazardous chemicals.

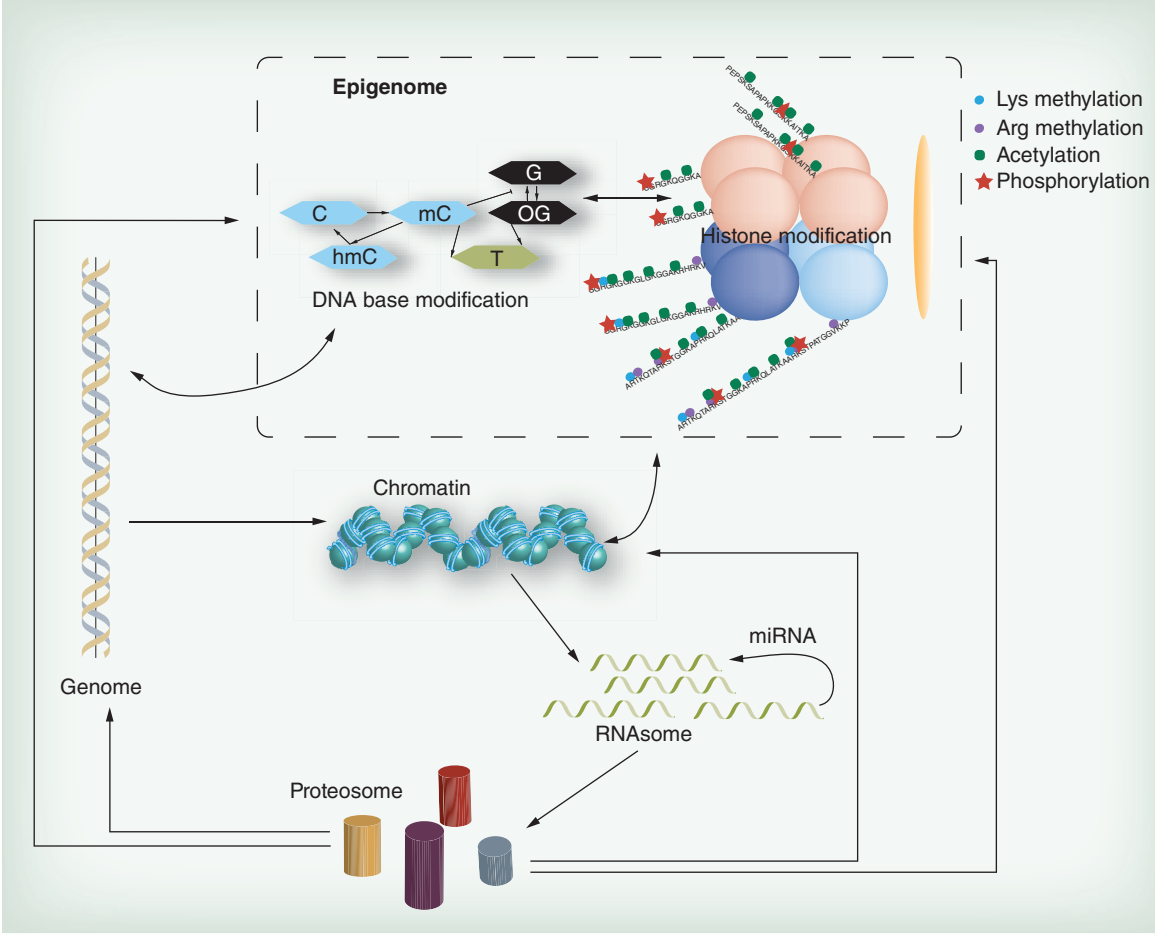


Figure 3. The organism as an information network. An organism includes multiple 'information-containing and transmitting systems and molecules', some of which may be inheritable. Broadly speaking, it can be seen as an interlocking network that includes the chromosomal and mitochondrial genomes (encoded by primary DNA sequences – mitochondrial not shown for reasons of space). The DNA of the genome plays host to some of the molecules that encode the epigenome. The epigenome consists of both modifications to individual DNA bases (usually cytosine or guanine) and modifications of the histone complex of the nucleosome (DNA omitted from histone complex diagram). Histone and DNA base modification can affect each other. Likewise DNA base modification can result in changes in the DNA primary sequence. The epigenome and genome together contribute to chromatin structure, and changes in chromatin structure can alter the likelihood of epigenomic modifications. Chromatin forms the basis for the RNAsome, which includes mRNA and regulatory molecules such as miRNA. The regulatory RNAs modify the processing of other RNAs, such as mRNA, and mRNA forms the template for translation to peptide primary sequences, which contribute heavily to the proteosome. The proteosome, itself, can modify any or all other levels, therein. The environment can, additionally, act upon any or all levels of the network, as well.

the DNA sequence, but it can be inherited [77]. Other specific instances of nongenetic inheritance of induced traits include paternal nutrition altering gene expression and health of offspring, particularly in adipose and pancreatic islet tissues and in expression of metabolic genes in general [78,79]. The CNS does not have a privileged immunity from nongenetic heritable alterations. Certain stress pathologies and responses have turned out to be epigenetic in nature and these changes are heritable [80,81]. Of particular interest, changes in brain function can be due to transgenerational epigenetic transmission [82], including neurobiological pathology [83–85] and 'behavioral' traits, such as aversion to acetophenone [86]. In ASD, for example, low paternal folate in diet [87], paternal age [42,88], grand paternal age [89] and paternal obesity but not maternal obesity [90] contribute to risk for ASD. While an argument may be made that effects such as grandpaternal age might be a genetic trait in that it might reflect a genetic propensity to reproduce later in life, linked to social traits common to ASD, factors such as paternal folate levels in diet would be more difficult to casually dismiss as 'genetic'. More work needs to be done to determine specific genetic/epigenetic/combined mechanisms of these intergenerational observations.

The paternal connection is particularly interesting because maternal conditions could influence ASD risk through well-known pre/perinatal pathways and not nongenetic inheritance. The majority of work relating intergenerational nongenetic effects to a neuropsychiatric disorder has been in ASD. It has been the presumption that the important environmental effects in late-life disorders such as PD and AD occur within the patient's own lifetime. Likewise, little specifically intergenerational epigenomic work has been performed in earlier-manifesting conditions such as schizophrenia or major depressive disorder. Nonetheless, some potential connections have been drawn for schizophrenia [91], depression and glucocorticoid sensitivity [92], and PD [93].

In short, tLEARn treats each person as a 'unit' that can accumulate preclinical or subclinical 'hits' as described in the original LEARn model [63] and these changes can then be passed along to offspring along with purely genetic (DNA primary sequence) changes. It is the transgenerational accumulation of hits that ultimately determines a sporadic disease state. For many people, few, if any, significant transgenerational hits would accompany their conception or gestation. For conditions that develop late in life, transgenerational effects are likely to be swamped by lifetime effects. LEARn is not so much replaced by as continued by tLEARn. Specifically, two people may undergo a set of potentially transmissible hits in their lifetimes. Neither of them accumulates enough hits and/or their hits do not accumulate before a critical pathogenic developmental cutoff. However, they are passed along through epigenomic inheritance. The child of these two people may thus have 'preaccumulated' sufficient hits to be at significant risk for a disorder (Figure 4). It must be stressed that the hits need not produce symptoms or microphenotypes in either parent to have a transgenerational effect. For example, epigenomic alterations that could direct the processing of the Alzheimer's associated amyloid-β precursor protein (APP) away from the Alzheimer's related amyloidogenic path and toward the 'anabolic' or α-processing pathway could actually be neuroprotective in adulthood. However, this specific instance is even more complex. Evidence exists that the anabolic pathway, in excess, may contribute to autism in early life. Specifically, the sAPPα product of the nonamyloidogenic processing of APP is neuroprotective and neuroproliferative [94,95]. Significantly higher levels of sAPPα have been reported in samples from autistic children versus nonautistic children [96,97], and particularly high APP levels may particularly correlate with increased aggression in autistic subjects [98].

Evidence for intergenerational transmission of traits via epigenetics

Prenatal exposure to dangerous chemicals and toxins can produce disease or increase the chance of disease in the fetus. How these changes may persist over generations has yet to be fully elucidated. Prenatal exposure to vinclozolin, a pesticide, can lead to impaired fertility in the F3 generation of the patriline [99]. However, further work has proved less clear-cut, and the original result has been both confirmed and failed to confirm [100,101]. Nevertheless, similar generational effects have also been observed after exposure to jet fuels, plastics, and other environmental toxins during gestation. These exposures seem to induce permanent epigenetic changes in the germline, and these heritable changes lead to adult-onset disease in subsequent generations without any further exposure to the insult. What is particularly interesting is that this process can apply to behavioral disorders, such as addiction and resistance to addiction [102–104]. Paternal cocaine use in mice prior to having offspring alters expression of DNA methyltransferases (*DNMT*) 1 and 3a in the seminiferous tubules and may lead to alterations in development of female offspring [105]. Such alteration of DNMT levels may be a means of transgenerational epigenetics.

Generational transmission of traits leading to conditions related to obesity and metabolic disease has some evidentiary support but is not conclusively demonstrated [106]. Alternate scenarios include environmental exposure altering developing germ cells within the developing fetus that are not manifest as epiphenotypes in F1 generation and effects induced into the fetus itself, which would be transmitted to the germline, affecting the next generation [107]. Recent evidence from mouse studies has shown that exposure of parents to environmental factors can have a profound effect on the changes in insulin sensitivity seen in Type 2 diabetes in offspring [108]. Generational effects have been seen in animal studies during which mice are reexposed to environmental stressors throughout several generations. Epigenetic models of obesity and the metabolic syndrome propose a programmed dysregulation of body weight in mice [109].

The experiences of one generation can seemingly alter the behavior of the subsequent offspring. The experience of maternal separation during early development altered DNA methylation levels in the brains and sperm of offspring and grandoffspring [110]. It has also been shown that there is a period of time during early gestation when stressors upon the dam, such as 36 h constant light, 15 min of predator odor, novel objects in the cage, overnight 5 min restraint in a 50-ml conical tube, novel white noise overnight, multiple cage

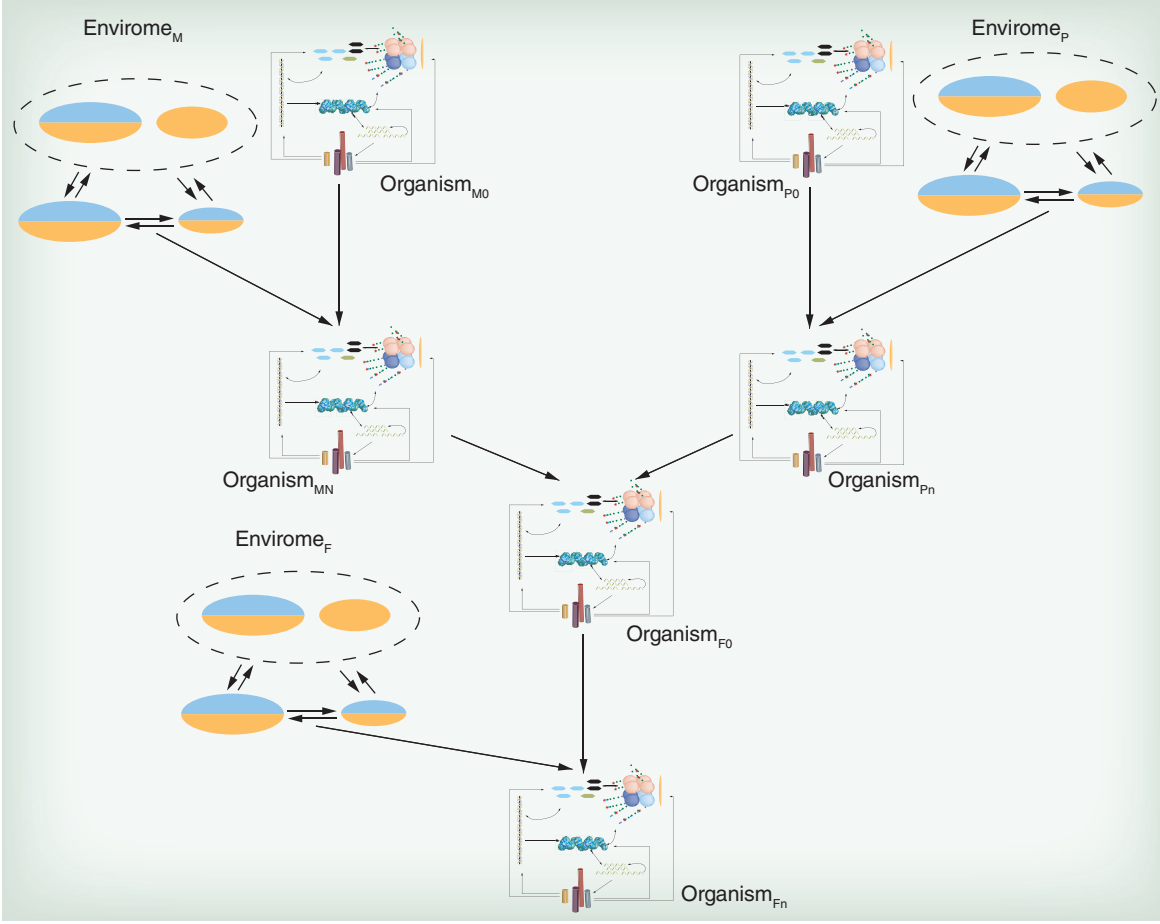


Figure 4. Intergenerational effects of enviromes on organisms: transgenerational latent early-life associated regulation. A simple one-generation pedigree is presented, in which the maternal envirome ($Envirome_M$) acts upon the initial maternal parent ($Organism_{M0}$) any number of times until time of reproduction/parturition ($Organism_{Mn}$), at which it makes its genetic and epigenetic contributions to the offspring ($Organism_{F0}$). The paternal counterparts ($Envirome_P$, $Organism_{P0}$ and $Organism_{Pn}$) likewise contribute in a similar fashion. Although not shown, the maternal contribution includes additional material, such as mitochondria, which would also be subject to environmental influence. The offspring, from conception, is then affected by the filial envirome ($Envirome_F$), which would include prenatal and perinatal conditions. Ultimately, this would give rise to the offspring at time 'n' ($Organism_{Fn}$), which could suffer a neuropathological condition. Although presented as distinct, the various enviromes can easily overlap to greater or lesser degree, and envirome–organism interactions are a multiple events.

changes and water-saturated bedding overnight can lead to dysmasculanization in the male offspring that recurs for several generations, and this is transmitted from the prenatally stressed males through their male offspring [111]. In adult male rats, increased anxiety, abnormal behavior and elevated corticosteroid levels were observed in both male and female offsprings of male mice after these mice underwent chronic social defeat paradigm. All offsprings were sired by *in vitro* fertilization. This permitted sperm to be collected from the defeat-exposed sires both before and after their exposure. Offspring from postdefeat-exposed sires had higher levels of anxiety/abnormal behavior and corticosteroids both in comparison to the same sires (predefeat) and no-exposed sires [112].

The role of these epigenetic changes on social behavior is critical for the understanding of these traits across generations, especially as related to disorders such as ASD and schizophrenia. These changes may also highly influence parenting style choices of mothers for future generations. Germline effects have been observed in fathers, hinting to their role in transmitting influence epigenetically to their offspring perhaps making up for a lack of parental care and influence often provided by the mother [113]. Transmission of gestational programming effects occurs in subsequent generations in the absence of continued adverse environmental exposures [114]. For example, the 'Dutch Famine' (1944–1945) cohort showed that prenatal exposure to famine resulted in hypomethylation of *IGF2* gene in whole blood, and

hypermethylation of two obesity-related nonimprinted genes (tumor necrosis factor, leptin) compared with same-sex siblings who had not been exposed to the famine [115,116]. In addition, increased adiposity was observed in the offspring of prenatally undernourished fathers from that cohort [117]. Understanding the mechanism of how traits are maintained and transmitted in the germline is the key to developing strategies to prevent environmental exposures from producing a disease phenotype across generations.

Executive summary

Genomic variations

- Small variants, copy number variants and rare variants do not add up to explain the cases of sporadic diseases.
- Most SNPs have little clinical value.
- Copy number variants and other structural variants contribute to genetic variation, and they are frequently found in both healthy people and those with 'sporadic' diseases.
- Rare genetic variants do not provide an explanation of over 90% cases of bipolar disorder, schizophrenia, or autism spectrum disorder.
- Genetic models can be greatly supported and expanded by explicit mechanistic explanations of gene–environment interaction.

Genetic variation on its own does not explain all pathogenesis of 'sporadic' disorders

- In many disorders with known genetic components, such as Alzheimer's disease, cases that can be explained solely by genetic mutation are a minor fraction.
- There is an important role played by epigenomic factors in conditions as diverse as Alzheimer's disease, Parkinson's disease, schizophrenia and even suicide.
- Epigenomic markers include modifications of DNA and of chromatin histones.
- Epigenomic contributions to neurobiological disorders may actually reach across generations.

Latent early-life associated regulation unites environment & gene expression

- For many sporadic disorders, the body accumulates 'hits' (that may include some genetic predispositions).
- No single hit is sufficient to cause disease.
- Each is individually latent.
- Many hits are of environmental origin and are 'recorded' in the organism through epigenomic markers.
- If a sufficient number of 'correct' hits occur before a critical developmental/aging threshold, the organism will develop the corresponding disorder

A model uniting environment, organism & generations: transgenerational latent early-life associated regulation

- In interaction with the environment, an organism exists on two levels.
- These are the information-coding and transmitting molecules and an information network that 'resides' in the 'media' of the molecules.
- An organism can also be seen as the substrate of the envirome.
- extends this across generations.
- Changes in brain function, such as aversion to acetophenone, can be due to transgenerational epigenetic transmission. In autism, for example, low paternal folate in diet, paternal age, grand paternal age and paternal obesity but not maternal obesity contribute to risk.

Evidence for intergenerational transmission of traits via epigenetics

- Exposures induce permanent epigenetic changes in the germline.
- Heritable changes lead to adult-onset disease in subsequent generations without further exposure to insult.
- This can apply to behavioral disorders, such as addiction and resistance to addiction.
- Mouse studies show that exposure of parents to environmental factors can have a profound effect on the changes in insulin sensitivity seen in Type 2 diabetes in offspring.
- The experiences of one generation can alter the behavior of subsequent offspring.
- Experience of maternal separation during early development altered DNA methylation levels in the brains and sperm of offspring and grandoffspring.

Conclusion & future perspective: research & treatment with transgenerational latent early-life associated regulation

- A longitudinal epigenome/envirome-wide association study would unite genetic sequence, epigenomic markers, environmental exposures, patient personal history at multiple time points and family history.
- Cohorts would include representatives of at least two generations, preferably more.
- The possibility exists that, should environmentally induced, inherited epigenomic 'mis-markings' increase risk for disorders in offspring, supplementing diet while avoiding repeating parental exposures may permanently reduce inherited risk.

Conclusion & future perspective: research & treatment based on the tLEARn concept

Testing tLEARn will require longitudinal, synthetic approaches. GWAS are end point assays that compare genetic sequences between diseased and nondiseased individuals. Several assays currently exist, which address parts of the question, but none consider the whole picture, particularly not in relation to changes over time. A longitudinal epigenome/envirome-wide association study (LEWAS) would unite genetic sequence, epigenomic markers, environmental exposures, patient personal history taken at multiple time points and family history. Ideally, cohorts would include representatives of at least two generations, preferably more. Several studies have partially implemented the LEWAS concept. Mouse studies have measured changes in specific gene-associated epigenetic changes to environment and concomitant changes in expression for those specific genes [118–120]. Targeted epigenomic surveys have been performed on human populations, comparing 'end point' differences in epigenomic markers and disease [63,121–123]. However, no study method currently unites multiple measurements over time with human populations and an envirome/organism-wide approach. Large-scale epigenomic surveys are currently underway, but these are end point, focused, without tracking changes in the epigenome [124,125]. The novel element of LEWAS is its longitudinal approach, attempting to measure, rather than infer *post hoc*, disease-critical organismal changes and relates those to previous environmental influences. LEWAS poses challenges in terms of scope and cost, particularly the need for large sample sizes in the face of unknown later development of disease and multiple sampling times. Multiple longitudinal studies have been done or are ongoing for conditions such as AD [126–129], PD [130,131], ASD [132–134], bipolar disorder [135,136], psychotic disorders [10,137] and other conditions [137], although not all are biochemical or epigenetic studies. Modern communication (especially the advent of social media and internet-based rating systems) has made these studies far more feasible than in the past. Methods exist to begin studies with very large sample sizes and reduce them to a 'hypothesis relevant' subsample [138]. For disorders of the CNS or other vital organs, direct sampling will not be possible. However, proxy tissues may be developed. For example, olfactory neuroepithelial cells are easily accessed and can be repeatedly sampled with safety. These have been adequate proxies for other neurological studies [139]. Also stem cell technology has advanced such that neuronal cells may be developed that reflect the genetic (and possibly epigenetic) characteristics of the donor. These can be modified with CRISPR-Cas 9-based methods.

Of course, one exciting possibility of tracing epigenomic antecedents to disorders is new treatment avenues, including the possibility of treatments that may not need to be lifelong. In particular, aberrant hypomethylation of DNA could potentially be reversed and this reversal could be maintained by means of avoiding high level or chronic exposures to materials such as pesticides and by eating a diet rich in materials like S-adenosyl-methionine (SAM). Dietary SAM supplementation via apple juice concentrate has been shown to reverse aberrant hypomethylation in AD-model mice [140]. Likewise, such supplementation also reversed cognitive deficits and reoriented DNA methylation in dietary-induced DNA hypomethylated mice [141]. It is worthwhile to explore the possibility that if specific environmentally-induced, inherited, epigenomic 'mis-markings' increase risk for sporadic disorders in offspring, perhaps supplementing diet while avoiding repeating parental exposures could permanently reduce inherited risk. Other ongoing studies exploring the epigenetics of dementias would provide potential preventative and therapeutic strategies [141]. Finally, as manifestations of the gut influence on behavior and diseases are beginning to be understood, future directions would further investigate the influence of gut microbiota [142] on tLEARn.

Financial & competing interests disclosure

The work was supported by grants from the National Institute on Aging, NIH R01-AG051086 and R21-AG042804; and Indiana Clinical & Translational Sciences Institute (ICTSI) and Indiana Spinal Cord and Brain Injury Research Fund (ISCBIRF); P30 AG010133 Indiana Alzheimer Disease Research Center, and R21 sub-award from Purdue University to DK Lahiri. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Argmann CA, Edwards JY, Sawyez CG *et al.* Regulation of macrophage cholesterol efflux through hydroxymethylglutaryl-CoA reductase inhibition: a role for

RhoA in ABCA1-mediated cholesterol efflux. *J. Biol. Chem.* 280(23), 22212–22221 (2005).

2 Nurnberger JI Jr, Koller DL, Jung J *et al.* Identification of pathways for bipolar disorder: a meta-analysis. *JAMA Psychiatry* 71(6), 657–664 (2014).

3 Samocha KE, Robinson EB, Sanders SJ *et al.* A framework for the interpretation of *de novo* mutation in human disease. *Nat. Genet.* 46(9), 944–950 (2014).

4 Rauch A, Wieczorek D, Graf E *et al.* Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* 380(9854), 1674–1682 (2012).

5 Gershon ES, Alliey-Rodriguez N. New ethical issues for genetic counseling in common mental disorders. *Am. J. Psychiatry* 170(9), 968–976 (2013).

6 Song C, Kanthasamy A, Anantharam V, Sun F, Kanthasamy AG. Environmental neurotoxic pesticide increases histone acetylation to promote apoptosis in dopaminergic neuronal cells: relevance to epigenetic mechanisms of neurodegeneration. *Mol. Pharmacol.* 77(4), 621–632 (2010).

7 Bahari-Javan S, Sananbenesi F, Fischer A. Histone-acetylation: a link between Alzheimer's disease and post-traumatic stress disorder? *Front. Neurosci.* 8, 160 (2014).

8 Veitch DP, Friedl KE, Weiner MW. Military risk factors for cognitive decline, dementia and Alzheimer's disease. *Curr. Alzheimer Res.* 10(9), 907–930 (2013).

9 Turecki G, Meaney MJ. Effects of the social environment and stress on glucocorticoid receptor gene methylation: a systematic review. *Biol. Psychiatry* 79(2), 87–96 (2016).

10 Schmitt A, Malchow B, Hasan A, Falkai P. The impact of environmental factors in severe psychiatric disorders. *Front. Neurosci.* 8, 19 (2014).

• **In this paper, the authors discuss the effect that several environmental agents have on neurodevelopmental processes. This article ties into the latent early-life associated regulation (LEARn) model and the effect of early exposure to stress and toxins on the development of the brain.**

11 Abdolmaleky HM, Smith CL, Faraone SV *et al.* Methylomics in psychiatry: modulation of gene-environment interactions may be through DNA methylation. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 127B(1), 51–59 (2004).

12 Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511(7510), 421–427 (2014).

13 Alzheimer's Association. 2014 Alzheimer's disease facts and figures. *Alzheimers Dement.* 10(2), e47–e92 (2014).

14 Narayan PJ, Lill C, Faull R, Curtis MA, Dragunow M. Increased acetyl and total histone levels in post-mortem Alzheimer's disease brain. *Neurobiol. Dis.* 74, 281–294 (2015).

15 Wang BY, Zhong Y, Zhao Z, Miao Y. Epigenetic suppression of hippocampal BDNF mediates the memory deficiency induced by amyloid fibrils. *Pharmacol. Biochem. Behav.* 126, 83–89 (2014).

16 Maloney B, Lahiri DK. Epigenetics of dementias: seeing dementia as a transformation rather than a condition. *Lancet Neurol.* (2016) (In press).

17 Feng Y, Jankovic J, Wu YC. Epigenetic mechanisms in Parkinson's disease. *J. Neurol. Sci.* 349(1–2), 3–9 (2015).

18 Pihlstrom L, Berge V, Rengmark A, Toft M. Parkinson's disease correlates with promoter methylation in the alpha-synuclein gene. *Mov. Disord.* 30(4), 577–580 (2015).

19 Haghighi F, Xin Y, Chanrion B *et al.* Increased DNA methylation in the suicide brain. *Dialogues Clin. Neurosci.* 16(3), 430–438 (2014).

20 Dempster EL, Wong CC, Lester KJ *et al.* Genome-wide methylomic analysis of monozygotic twins discordant for adolescent depression. *Biol. Psychiatry* 76(12), 977–983 (2014).

21 McGowan PO, Sasaki A, Huang TC *et al.* Promoter-wide hypermethylation of the ribosomal RNA gene promoter in the suicide brain. *PLoS ONE* 3(5), e2085 (2008).

22 Zhu L, Wang X, Li XL *et al.* Epigenetic dysregulation of SHANK3 in brain tissues from individuals with autism spectrum disorders. *Hum. Mol. Genet.* 23(6), 1563–1578 (2014).

23 Ladd-Acosta C, Hansen KD, Briem E, Fallin MD, Kaufmann WE, Feinberg AP. Common DNA methylation alterations in multiple brain regions in autism. *Mol. Psychiatry* 19(8), 862–871 (2013).

24 Wong CC, Meaburn EL, Ronald A *et al.* Methylomic analysis of monozygotic twins discordant for autism spectrum disorder and related behavioural traits. *Mol. Psychiatry* 19(4), 495–503 (2013).

25 Siniscalco D, Cirillo A, Bradstreet JJ, Antonucci N. Epigenetic findings in autism: new perspectives for therapy. *Int. J. Environ. Res. Public Health* 10(9), 4261–4273 (2013).

26 Alanazi AS. The role of nutraceuticals in the management of autism. *Saudi Pharm. J.* 21(3), 233–243 (2013).

27 Yasuda H, Tsutsui T. Assessment of infantile mineral imbalances in autism spectrum disorders (ASDs). *Int. J. Environ. Res. Public Health* 10(11), 6027–6043 (2013).

28 Mastroeni D, McKee A, Grover A, Rogers J, Coleman PD. Epigenetic differences in cortical neurons from a pair of monozygotic twins discordant for Alzheimer's disease. *PLoS ONE* 4(8), e6617 (2009).

29 Chouliaras L, Mastroeni D, Delvaux E *et al.* Consistent decrease in global DNA methylation and hydroxymethylation in the hippocampus of Alzheimer's disease patients. *Neurobiol. Aging* 34(9), 2091–2099 (2013).

30 Kaminsky Z, Jones I, Verma R *et al.* DNA methylation and expression of KCNQ3 in bipolar disorder. *Bipolar Disord.* 17(2), 150–159 (2015).

31 Guidotti A, Grayson DR. DNA methylation and demethylation as targets for antipsychotic therapy. *Dialogues Clin. Neurosci.* 16(3), 419–429 (2014).

32 Li Y, Camarillo C, Xu J *et al.* Genome-wide methylome analyses reveal novel epigenetic regulation patterns in schizophrenia and bipolar disorder. *BioMed. Res. Int.* 2015, 201587 (2015).

33 Kundakovic M, Gudsnuk K, Herbstman JB, Tang D, Perera FP, Champagne FA. DNA methylation of BDNF as

a biomarker of early-life adversity. *Proc. Natl Acad. Sci. USA* 112(22), 6807–6813 (2014).

34 Dell'osso B, D'addario C, Carlotta Palazzo M *et al.* Epigenetic modulation of BDNF gene: differences in DNA methylation between unipolar and bipolar patients. *J. Affect. Disord.* 166, 330–333 (2014).

35 Kouzarides T. Chromatin modifications and their function. *Cell* 128(4), 693–705 (2007).

36 Baltazar MT, Dinis-Oliveira RJ, De Lourdes Bastos M, Tsatsakis AM, Duarte JA, Carvalho F. Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative diseases: a mechanistic approach. *Toxicol. Lett.* 230(2), 85–103 (2014).

37 Song C, Kanthasamy A, Jin H, Anantharam V, Kanthasamy AG. Paraquat induces epigenetic changes by promoting histone acetylation in cell culture models of dopaminergic degeneration. *Neurotoxicology* 32(5), 586–595 (2011).

38 Kanthasamy A, Jin H, Anantharam V *et al.* Emerging neurotoxic mechanisms in environmental factors-induced neurodegeneration. *Neurotoxicology* 33(4), 833–837 (2012).

39 Jin H, Kanthasamy A, Harischandra DS *et al.* Histone hyperacetylation up-regulates protein kinase C delta in dopaminergic neurons to induce cell death: relevance to epigenetic mechanisms of neurodegeneration in Parkinson disease. *J. Biol. Chem.* 289(50), 34743–34767 (2014).

40 Covington HE 3rd, Maze I, Vialou V, Nestler EJ. Antidepressant action of HDAC inhibition in the prefrontal cortex. *Neuroscience* 298, 329–335 (2015).

41 Walker DM, Cates HM, Heller EA, Nestler EJ. Regulation of chromatin states by drugs of abuse. *Curr. Opin. Neurobiol.* 30, 112–121 (2015).

42 D'onofrio BM, Rickert ME, Frans E *et al.* Paternal age at childbearing and offspring psychiatric and academic morbidity. *JAMA Psychiatry* 71(4), 432–438 (2014).

43 Jenkins TG, Aston KI, Pflueger C, Cairns BR, Carrell DT. Age-associated sperm DNA methylation alterations: possible implications in offspring disease susceptibility. *PLoS Genet.* 10(7), e1004458 (2014).

- **This article discusses the impact of age-associated epigenetic changes on genes associated with schizophrenia and bipolar disorder. Work of this article is directly related to the transgenerational part of the transgenerational latent early-life associated regulation (tLEARn) concept in that these epigenetic changes in the father are manifested in the sperm and potentially lead to changes in offspring.**

44 Wang SH, Liu CM, Hwu HG, Hsiao CK, Chen WJ. Association of older paternal age with earlier onset among co-affected schizophrenia sib-pairs. *Psychol. Med.* doi:10.1017/S0033291715000203 (2015) (Epub ahead of print).

45 Bell CG, Beck S. The epigenomic interface between genome and environment in common complex diseases. *Brief. Funct. Genomics* 9, 477–485 (2010).

46 Migliore L, Coppede F. Genetics, environmental factors and the emerging role of epigenetics in neurodegenerative diseases. *Mutat. Res.* 667(1–2), 82–97 (2009).

47 Trollope AF, Gutierrez-Mecinas M, Mifsud KR, Collins A, Saunderson EA, Reul JM. Stress, epigenetic control of gene expression and memory formation. *Exp. Neurol.* 233(1), 3–11 (2012).

48 Long JM, Lahiri DK. Current drug targets for modulating Alzheimer's amyloid precursor protein: role of specific micro-RNA species. *Curr. Med. Chem.* 18(22), 3314–3321 (2011).

49 Bao N, Lye KW, Barton MK. MicroRNA binding sites in *Arabidopsis* class III HD-ZIP mRNAs are required for methylation of the template chromosome. *Dev. Cell* 7(5), 653–662 (2004).

50 Tuddenham L, Wheeler G, Ntounia-Fousara S *et al.* The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. *FEBS Lett.* 580(17), 4214–4217 (2006).

51 Asada K, Nakajima T, Shimazu T *et al.* Demonstration of the usefulness of epigenetic cancer risk prediction by a multicentre prospective cohort study. *Gut* 64(3), 388–396 (2015).

52 Szulwach KE, Li X, Smrt RD *et al.* Cross talk between microRNA and epigenetic regulation in adult neurogenesis. *J. Cell Biol.* 189(1), 127–141 (2010).

- **This article explains the interaction of miRNAs and epigenetic pathways as related to neurogenesis. Demonstration of this relationship is important in the discussion of epigenetic mechanisms discussed in the tLEARn paper.**

53 Cimmino L, Abdel-Wahab O, Levine RL, Aifantis I. TET family proteins and their role in stem cell differentiation and transformation. *Cell Stem Cell* 9(3), 193–204 (2011).

54 Fabbri M, Garzon R, Cimmino A *et al.* MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc. Natl Acad. Sci. USA* 104(40), 15805–15810 (2007).

55 Long LM, He BF, Huang GQ, Guo YH, Liu YS, Huo JR. MicroRNA-214 functions as a tumor suppressor in human colon cancer via the suppression of ADP-ribosylation factor-like protein 2. *Oncol. Lett.* 9(2), 645–650 (2015).

56 Izzotti A, Calin GA, Arrigo P, Steele VE, Croce CM, De Flora S. Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke. *FASEB J.* 23(3), 806–812 (2009).

57 Radom-Aizik S, Zaldivar F Jr, Oliver S, Galassetti P, Cooper DM. Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes. *J. Appl. Physiol. (1985)* 109(1), 252–261 (2010).

58 Bollati V, Marinelli B, Apostoli P *et al.* Exposure to metal-rich particulate matter modifies the expression of candidate microRNAs in peripheral blood leukocytes. *Environ. Health Perspect.* 118(6), 763–768 (2010).

59 Tang X, Wen S, Zheng D *et al.* Acetylation of drosha on the N-terminus inhibits its degradation by ubiquitination. *PLoS ONE* 8(8), e72503 (2013).

60 Liu Y, Studzinski C, Beckett T, Murphy MP, Klein RL, Hersh LB. Circulating neprilysin clears brain amyloid. *Mol. Cell. Neurosci.* 45(2), 101–107 (2010).

61 Koumangoye RB, Andl T, Taubenslag KJ *et al.* SOX4 interacts with EZH2 and HDAC3 to suppress microRNA-31 in invasive esophageal cancer cells. *Mol. Cancer* 14, 24 (2015).

62 Hwang JY, Kaneko N, Noh KM, Pontarelli F, Zukin RS. The gene silencing transcription factor REST represses miR-132

expression in hippocampal neurons destined to die. *J. Mol. Biol.* 426(20), 3454–3466 (2014).

63 Lahiri DK, Maloney B, Zawia NH. The LEARn model: an epigenetic explanation for idiopathic neurobiological diseases. *Mol. Psychiatry* 14(11), 992–1003 (2009).

- **The basis of the tLEARn concept, the LEARn model explains and defines somatic epitype and the relationship between gene and environment as related to disease latency and epigenetic regulation.**

64 Maloney B, Sambamurti K, Zawia N, Lahiri DK. Applying epigenetics to Alzheimer's disease via the latent early-life associated regulation (LEARn) model. *Curr. Alzheimer Res.* 9(5), 589–599 (2012).

- **This paper specifically applies the LEARn model to a neurodegenerative disease (AD).**

65 Vassiliev ON. Formulation of the multi-hit model with a non-Poisson distribution of hits. *Int. J. Radiat. Oncol. Biol. Phys.* 83(4), 1311–1316 (2012).

66 Teixeira AP, Dias JM, Carinhas N *et al.* Cell functional enviromics: unravelling the function of environmental factors. *BMC Syst. Biol.* 5, 92 (2011).

67 Lahiri DK, Maloney B. Gene x environment interaction by a longitudinal epigenome-wide association study (LEWAS) overcomes limitations of genome-wide association study (GWAS). *Epigenomics* 4(6), 685–699 (2012).

68 Wild CP. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol. Biomarkers Prev.* 14(8), 1847–1850 (2005).

- **This article highlights the concept of an exposome and the increasing need for environmental risk factors to be considered when understanding the development of chronic disease. This is an important aspect of the tLEARn concept.**

69 Coley RL, Carrano J, Lewin-Bizan S. Unpacking links between fathers' antisocial behaviors and children's behavior problems: direct, indirect, and interactive effects. *J. Abnorm. Child Psychol.* 39(6), 791–804 (2011).

70 Saile R, Ertl V, Neuner F, Catani C. Does war contribute to family violence against children? Findings from a two-generational multi-informant study in Northern Uganda. *Child Abuse Negl.* 38(1), 135–146 (2014).

71 Barnett MA, Scaramella LV, Neppl TK, Ontai LL, Conger RD. Grandmother involvement as a protective factor for early childhood social adjustment. *J. Fam. Psychol.* 24(5), 635–645 (2010).

72 Jaffee SR, Bowes L, Ouellet-Morin I *et al.* Safe, stable, nurturing relationships break the intergenerational cycle of abuse: a prospective nationally representative cohort of children in the United Kingdom. *J. Adolesc. Health* 53(Suppl. 4), S4–S10 (2013).

73 Conger RD, Schofield TJ, Neppl TK, Merrick MT. Disrupting intergenerational continuity in harsh and abusive parenting: the importance of a nurturing relationship with a romantic partner. *J. Adolesc. Health* 53(Suppl. 4), S11–S17 (2013).

74 Farrugia D. Exploring stigma: medical knowledge and the stigmatisation of parents of children diagnosed with autism spectrum disorder. *Sociol. Health Illn.* 31(7), 1011–1027 (2009).

75 Lidsky TI. Is the aluminum hypothesis dead? *J. Occup. Environ. Med.* 56(Suppl. 5), S73–S79 (2014).

76 Seeman MV. The changing role of mother of the mentally ill: from schizophrenogenic mother to multigenerational caregiver. *Psychiatry* 72(3), 284–294 (2009).

77 Pentinat T, Ramon-Krauel M, Cebria J, Diaz R, Jimenez-Chillaron JC. Transgenerational inheritance of glucose intolerance in a mouse model of neonatal overnutrition. *Endocrinology* 151(12), 5617–5623 (2010).

78 Ng SF, Lin RC, Maloney CA, Youngson NA, Owens JA, Morris MJ. Paternal high-fat diet consumption induces common changes in the transcriptomes of retroperitoneal adipose and pancreatic islet tissues in female rat offspring. *FASEB J.* 28(4), 1830–1841 (2014).

79 Carone BR, Fauquier L, Habib N *et al.* Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* 143(7), 1084–1096 (2010).

- **This work demonstrates that the paternal diet can in fact transgenerationally affect metabolism in offspring, which supports the tLEARn model characteristic of a 'reprogramming of the heritable epigenome.'**

80 Crews D, Gillette R, Scarpino SV, Manikkam M, Savenkova MI, Skinner MK. Epigenetic transgenerational inheritance of altered stress responses. *Proc. Natl Acad. Sci. USA* 109(23), 9143–9148 (2012).

81 Matthews SG, Phillips DI. Transgenerational inheritance of stress pathology. *Exp. Neurol.* 233(1), 95–101 (2012).

82 Bohacek J, Gapp K, Saab BJ, Mansuy IM. Transgenerational epigenetic effects on brain functions. *Biol. Psychiatry* 73(4), 313–320 (2013).

83 Bohacek J, Mansuy IM. Epigenetic inheritance of disease and disease risk. *Neuropsychopharmacology* 38(1), 220–236 (2013).

- **This review article eloquently describes the ways that epigenetic modifications can be changed by environmental factors in life and have effects across generations. This is a key concept in the tLEARn paper.**

84 Jiang YH, Sahoo T, Michaelis RC *et al.* A mixed epigenetic/genetic model for oligogenic inheritance of autism with a limited role for UBE3A. *Am. J. Med. Genet. A* 131(1), 1–10 (2004).

85 Saab BJ, Mansuy IM. Neurobiological disease etiology and inheritance: an epigenetic perspective. *J. Exp. Biol.* 217(Pt 1), 94–101 (2014).

86 Dias BG, Ressler KJ. Parental olfactory experience influences behavior and neural structure in subsequent generations. *Nat. Neurosci.* 17(1), 89–96 (2014).

87 Lambrot R, Xu C, Saint-Phar S *et al.* Low paternal dietary folate alters the mouse sperm epigenome and is associated with negative pregnancy outcomes. *Nat. Commun.* 4, 2889 (2013).

88 Mcgrath JJ, Petersen L, Agerbo E, Mors O, Mortensen PB, Pedersen CB. A comprehensive assessment of parental age and psychiatric disorders. *JAMA Psychiatry* 71(3), 301–309 (2014).

89 Sampino S, Juszczak GR, Zacchini F *et al.* Grand-paternal age and the development of autism-like symptoms in mice progeny. *Transl. Psychiatry* 4, e386 (2014).

90 Suren P, Gunnes N, Roth C *et al.* Parental obesity and risk of autism spectrum disorder. *Pediatrics* 133(5), e1128–e1138 (2014).

91 Bygren LO. Intergenerational health responses to adverse and enriched environments. *Annu. Rev. Public Health* 34, 49–60 (2013).

92 Lehrner A, Bierer LM, Passarelli V *et al.* Maternal PTSD associates with greater glucocorticoid sensitivity in offspring of Holocaust survivors. *Psychoneuroendocrinology* 40, 213–220 (2014).

93 Laffita-Mesa JM, Bauer PO, Kouri V *et al.* Epigenetics DNA methylation in the core ataxin-2 gene promoter: novel physiological and pathological implications. *Hum. Genet.* 131(4), 625–638 (2012).

94 Milosch N, Tanriover G, Kundu A *et al.* Holo-APP and G-protein-mediated signaling are required for sAPPalpha-induced activation of the Akt survival pathway. *Cell Death Dis.* 5, e1391 (2014).

95 Corrigan F, Vink R, Blumbergs PC, Masters CL, Cappai R, Van Den Heuvel C. Evaluation of the effects of treatment with sAPPalpha on functional and histological outcome following controlled cortical impact injury in mice. *Neurosci. Lett.* 515(1), 50–54 (2012).

96 Lahiri DK, Sokol DK, Erickson C, Ray B, Ho CY, Maloney B. Autism as early neurodevelopmental disorder: evidence for an sAPPalpha-mediated anabolic pathway. *Front. Cell. Neurosci.* 7, 94 (2013).

97 Ray B, Long JM, Sokol DK, Lahiri DK. Increased secreted amyloid precursor protein-alpha (sAPPalpha) in severe autism: proposal of a specific, anabolic pathway and putative biomarker. *PLoS ONE* 6(6), e20405 (2011).

98 Sokol DK, Chen D, Farlow MR *et al.* High levels of Alzheimer beta-amyloid precursor protein (APP) in children with severely autistic behavior and aggression. *J. Child Neurol.* 21(6), 444–449 (2006).

99 Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 308(5727), 1466–1469 (2005).

100 Clement TM, Savenkova MI, Settles M, Anway MD, Skinner MK. Alterations in the developing testis transcriptome following embryonic vinclozolin exposure. *Reprod. Toxicol.* 30(3), 353–364 (2010).

101 Schneider S, Kaufmann W, Buesen R, Van Ravenzwaay B. Vinclozolin – the lack of a transgenerational effect after oral maternal exposure during organogenesis. *Reprod. Toxicol.* 25(3), 352–360 (2008).

102 Vassoler FM, Sadri-Vakili G. Mechanisms of transgenerational inheritance of addictive-like behaviors. *Neuroscience* 264, 198–206 (2014).

103 Vassoler FM, White SL, Schmidt HD, Sadri-Vakili G, Pierce RC. Epigenetic inheritance of a cocaine-resistance phenotype. *Nat. Neurosci.* 16(1), 42–47 (2013).

104 Sadri-Vakili G, Kumaresan V, Schmidt HD *et al.* Cocaine-induced chromatin remodeling increases brain-derived neurotrophic factor transcription in the rat medial prefrontal cortex, which alters the reinforcing efficacy of cocaine. *J. Neurosci.* 30(35), 11735–11744 (2010).

105 He F, Lidow IA, Lidow MS. Consequences of paternal cocaine exposure in mice. *Neurotoxicol. Teratol.* 28(2), 198–209 (2006).

106 Stegemann R, Buchner DA. Transgenerational inheritance of metabolic disease. *Semin. Cell Dev. Biol.* 43, 131–140 (2015).

107 Drake AJ, Liu L. Intergenerational transmission of programmed effects: public health consequences. *Trends Endocrinol. Metab.* 21(4), 206–213 (2010).

108 Raciti GA, Longo M, Parrillo L *et al.* Understanding Type 2 diabetes: from genetics to epigenetics. *Acta Diabetol.* 52(5), 821–827 (2015).

109 Plagemann A, Harder T, Brunn M *et al.* Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. *J. Physiol.* 587(Pt 20), 4963–4976 (2009).

110 Franklin TB, Linder N, Russig H, Thony B, Mansuy IM. Influence of early stress on social abilities and serotonergic functions across generations in mice. *PLoS ONE* 6(7), e21842 (2011).

111 Morgan CP, Bale TL. Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. *J. Neurosci.* 31(33), 11748–11755 (2011).

112 Dietz DM, Laplant Q, Watts EL *et al.* Paternal transmission of stress-induced pathologies. *Biol. Psychiatry* 70(5), 408–414 (2011).

113 Champagne FA. Early environments, glucocorticoid receptors, and behavioral epigenetics. *Behav. Neurosci.* 127(5), 628–636 (2013).

114 Desai M, Jellyman JK, Ross MG. Epigenomics, gestational programming and risk of metabolic syndrome. *Int. J. Obes. (Lond.)* 39(4), 633–641 (2015).

115 Heijmans BT, Tobi EW, Stein AD *et al.* Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc. Natl Acad. Sci. USA* 105(44), 17046–17049 (2008).

116 Tobi EW, Lumey LH, Talens RP *et al.* DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum. Mol. Genet.* 18(21), 4046–4053 (2009).

117 Veenendaal MV, Painter RC, De Rooij SR *et al.* Transgenerational effects of prenatal exposure to the 1944–45 Dutch famine. *BJOG* 120(5), 548–553 (2013).

118 Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc. Natl Acad. Sci. USA* 104(32), 13056–13061 (2007).

119 Branchi I, Karpova NN, D'andrea I, Castren E, Alleva E. Epigenetic modifications induced by early enrichment are associated with changes in timing of induction of BDNF expression. *Neurosci. Lett.* 495(3), 168–172 (2011).

120 Vucetic Z, Kimmel J, Reyes TM. Chronic high-fat diet drives postnatal epigenetic regulation of mu-opioid receptor

in the brain. *Neuropsychopharmacology* 36(6), 1199–1206 (2011).

121 Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. *Nat. Rev. Genet.* 8(4), 253–262 (2007).

122 Heindel JJ, Mcallister KA, Worth L Jr, Tyson FL. Environmental epigenomics, imprinting and disease susceptibility. *Epigenetics* 1(1), 1–6 (2006).

123 Sacco A, Issa GC, Zhang Y *et al*. Epigenetic modifications as key regulators of Waldenstrom's Macroglobulinemia biology. *J. Hematol. Oncol.* 3(1), 38 (2010).

124 American Association for Cancer Research Human Epigenome Task Force & European Union, Network of Excellence, Scientific Advisory Board. Moving AHEAD with an international human epigenome project. *Nature* 454(7205), 711–715 (2008).

125 Brena RM, Huang TH, Plass C. Toward a human epigenome. *Nat. Genet.* 38(12), 1359–1360 (2006).

126 Yu L, Chibnik LB, Srivastava GP *et al.* Association of Brain DNA methylation in *SORL1*, *ABCA7*, *HLA-DRB5*, *SLC24A4*, and *BIN1* with pathological diagnosis of Alzheimer disease. *JAMA Neurol.* 72(1), 15–24 (2015).

127 Marioni RE, Shah S, Mcrae AF *et al.* The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth Cohort 1936. *Int. J. Epidemiol.* 44(4), 1388–1396 (2015).

128 Exalto LG, Van Der Flier WM, Van Boheemen CJ *et al.* The metabolic syndrome in a memory clinic population: relation with clinical profile and prognosis. *J. Neurol. Sci.* 351(1–2), 18–23 (2015).

129 Marioni RE, Shah S, McRae AF *et al.* DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol.* 16, 25 (2015).

130 Reetz K, Dogan I, Costa AS *et al.* Biological and clinical characteristics of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) cohort: a cross-sectional analysis of baseline data. *Lancet Neurol.* 14(2), 174–182 (2015).

131 Abbruzzese G, Barone P, Ceravolo R *et al.* Clinical variables associated with treatment changes in Parkinson's disease: results from the longitudinal phase of the REASON study. *Neurol. Sci.* 36(6), 935–943 (2015).

132 Helles A, Gillberg CI, Gillberg C, Billstedt E. Asperger syndrome in males over two decades: stability and predictors of diagnosis. *J. Child Psychol. Psychiatry* 56(6), 711–718 (2015).

133 Woodman AC, Smith LE, Greenberg JS, Mailick MR. Change in autism symptoms and maladaptive behaviors in adolescence and adulthood: the role of positive family processes. *J. Autism Dev. Disord.* 45(1), 111–126 (2015).

134 Hernandez RN, Feinberg RL, Vaurio R, Passanante NM, Thompson RE, Kaufmann WE. Autism spectrum disorder in fragile X syndrome: a longitudinal evaluation. *Am. J. Med. Genet. A* 149A(6), 1125–1137 (2009).

135 Seleem MA, Merranko JA, Goldstein TR *et al.* The longitudinal course of sleep timing and circadian preferences in adults with bipolar disorder. *Bipolar Disord.* 17(4), 392–402 (2015).

136 Nurnberger JI Jr, Mcinnis M, Reich W *et al.* A high-risk study of bipolar disorder. Childhood clinical phenotypes as precursors of major mood disorders. *Arch. Gen. Psychiatry* 68(10), 1012–1020 (2011).

137 Savill M, Banks C, Khanom H, Priebe S. Do negative symptoms of schizophrenia change over time? A meta-analysis of longitudinal data. *Psychol. Med.* 45(8), 1613–1627 (2015).

138 Ferguson CJ. Is psychological research really as good as medical research? Effect size comparisons between psychology and medicine. *Rev. Gen. Psychol.* 13(2), 130–136 (2009).

139 Perry C, Mackay-Sim A, Feron F, Mcgrath J. Olfactory neural cells: an untapped diagnostic and therapeutic resource. The 2000 Ogura Lecture. *Laryngoscope* 112(4), 603–607 (2002).

140 Chan A, Shea TB. Supplementation with apple juice attenuates presenilin-1 overexpression during dietary and genetically-induced oxidative stress. *J. Alzheimers Dis.* 10(4), 353–358 (2006).

141 Rogers EJ, Milhalik S, Orthiz D, Shea TB. Apple juice prevents oxidative stress and impaired cognitive performance caused by genetic and dietary deficiencies in mice. *J. Nutr. Health Aging* 8(2), 92–97 (2004).

142 De Palma G, Blennerhassett P, Lu J *et al*. Microbiota and host determinants of behaviouralphenotype in maternally separated mice. *Nat.Commun.* 6, 7735 (2015).